=> fil .bec

COST IN U.S. DOLLARS
SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST
SINCE FILE TOTAL
O.21
O.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS, ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 16:47:54 ON 09 JAN 2004 ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

#### 11 FILES IN THE FILE LIST

=> s (commercial or scale or batch)(10a)(sialyl? or glycosylat?)

FILE 'MEDLINE'

38986 COMMERCIAL

120028 SCALE

9420 BATCH

6543 SIALYL?

39942 GLYCOSYLAT?

L1 56 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)

## FILE 'SCISEARCH'

87545 COMMERCIAL

278198 SCALE

33844 BATCH

6705 SIALYL?

30960 GLYCOSYLAT?

L2 88 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)

### FILE 'LIFESCI'

21315 COMMERCIAL

32165 SCALE

10276 BATCH

1615 SIALYL?

9593 GLYCOSYLAT?

L3 27 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)

# FILE 'BIOTECHDS'

5741 COMMERCIAL

14335 SCALE

12107 BATCH

403 SIALYL?

3526 GLYCOSYLAT?

L4 66 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)

### FILE 'BIOSIS'

85230 COMMERCIAL

139389 SCALE

22994 BATCH

7315 SIALYL?

34885 GLYCOSYLAT?

L5 73 (COMMERCIAL OR SCALE OR BATCH)(10A)(SIALYL? OR GLYCOSYLAT?)

## FILE 'EMBASE'

36480 COMMERCIAL

131776 SCALE

15222 BATCH

6182 SIALYL?

31840 GLYCOSYLAT?

L6 75 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)

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FILE 'HCAPLUS'
        28321 COMMERCIAL
        266795 COM
        280438 COMMERCIAL
                 (COMMERCIAL OR COM)
        314173 SCALE
         78225 BATCH
          8086 SIALYL?
         37716 GLYCOSYLAT?
           144 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)
L7
FILE 'NTIS'
         51928 COMMERCIAL
         80947 SCALE
          6305 BATCH
            18 SIALYL?
           118 GLYCOSYLAT?
             1 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)
L8
FILE 'ESBIOBASE'
         20071 COMMERCIAL
         49588 SCALE
         10033 BATCH
          2703 SIALYL?
         11895 GLYCOSYLAT?
            41 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)
L9
FILE 'BIOTECHNO'
         14938 COMMERCIAL
         23003 SCALE
         11409 BATCH
          3202 SIALYL?
         16990 GLYCOSYLAT?
            46 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)
L10
FILE 'WPIDS'
         39606 COMMERCIAL
        117188 SCALE
         26215 BATCH
           410 SIALYL?
          2412 GLYCOSYLAT?
            12 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)
L11
TOTAL FOR ALL FILES
           629 (COMMERCIAL OR SCALE OR BATCH) (10A) (SIALYL? OR GLYCOSYLAT?)
=> s 112 not 1998-2004/py
FILE 'MEDLINE'
       2979269 1998-2004/PY
           29 L1 NOT 1998-2004/PY
L13
FILE 'SCISEARCH'
       5861045 1998-2004/PY
L14
            45 L2 NOT 1998-2004/PY
FILE 'LIFESCI'
        617203 1998-2004/PY
L15
           15 L3 NOT 1998-2004/PY
FILE 'BIOTECHDS'
        103353 1998-2004/PY
           47 L4 NOT 1998-2004/PY
L16
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FILE 'BIOSIS'

3254024 1998-2004/PY

L17 42 L5 NOT 1998-2004/PY

FILE 'EMBASE'

2634784 1998-2004/PY

L18 44 L6 NOT 1998-2004/PY

FILE 'HCAPLUS'

5491477 1998-2004/PY

L19 74 L7 NOT 1998-2004/PY

FILE 'NTIS'

117194 1998-2004/PY

L20 1 L8 NOT 1998-2004/PY

FILE 'ESBIOBASE'

1701387 1998-2004/PY

L21 12 L9 NOT 1998-2004/PY

FILE 'BIOTECHNO'

724097 1998-2004/PY

L22 24 L10 NOT 1998-2004/PY

FILE 'WPIDS'

4772772 1998-2004/PY

L23 3 L11 NOT 1998-2004/PY

TOTAL FOR ALL FILES

L24 336 L12 NOT 1998-2004/PY

=> dup rem 124

PROCESSING COMPLETED FOR L24

L25 156 DUP REM L24 (180 DUPLICATES REMOVED)

=> d tot

L25 ANSWER 1 OF 156 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

- TI Control of interferon-gamma **glycosylation** by the addition of defined lipid supplements to **batch** cultures of recombinant Chinese hamster ovary cells.
- Funatsu, K. [Editor]; Shirai, Y. [Editor]; Matsushita, T. [Editor]. (1997) pp. 339-345. Animal Cell Technology. Publisher: Kluwer Academic Publishers, PO Box 989, 3300 AZ Dordrecht, Netherlands; Kluwer Academic Publishers, 101 Phillip Drive, Norwell; Massachusetts 02061, USA. Series: Animal Cell Technology. Meeting Info.: Eighth Annual Meeting of the Japanese Association for Animal Cell Technology. Iizuka, Japan. November 6-10, 1995.
- AU Green, N. H. [Reprint author]; Hooker, A. D. [Reprint author]; James, D. C. [Reprint author]; Baines, A. J. [Reprint author]; Strange, P. G.; Jenkins, N.; Bull, A. T. [Reprint author]
- AN 1997:466359 BIOSIS
- L25 ANSWER 2 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
- TI Chemoenzymatic synthesis of GM3, Lewis-X and sialyl-Lewis-X oligosaccharides in 13C-enriched form;

ganglioside-GM3 oligosaccharide etc. production by sialylation with Trypanosoma cruzi recombinant trans-sialidase, and fucosylation with milk fucosyltransferase

SO Tetrahedron Lett.; (1997) 38, 33, 5861-64 CODEN: TELEAY ISSN: 0040-4039

AU Probert M A; Milton M J; Harris R; Schenkman S; Brown J M; Homans S W; \*Field R A

AN 1997-10511 BIOTECHDS

- L25 ANSWER 3 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 1
- Gamma-interferon production and quality in stoichiometric fed-batch cultures of Chinese hamster ovary (CHO) cells under serum-free conditions BIOTECHNOLOGY AND BIOENGINEERING, (5 DEC 1997) Vol. 56, No. 5, pp.
- SO BIOTECHNOLOGY AND BIOENGINEERING, (5 DEC 1997) Vol. 56, No. 5, pp 577-582.
  - Publisher: JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012. ISSN: 0006-3592.
- AU Xie L Z; Nyberg G; Gu X J; Li H Y; Mollborn F; Wang D I C (Reprint)
- AN 97:814754 SCISEARCH
- L25 ANSWER 4 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
- TI Production of recombinant proteins in transgenic plants: practical considerations;

a review

- SO Biotechnol Bioeng; (1997) 56, 5, 473-84 CODEN: BIBIAU ISSN: 0006-3592
- AU Kusnadi A R; \*Nikolov Z L; Howard J A
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- L25 ANSWER 5 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 2
- TI Production of recombinant human antithrombin III on 20-L bioreactor scale: Correlation of supernatant neuraminidase activity, desialylation, and decrease of biological activity of recombinant glycoprotein
- SO BIOTECHNOLOGY AND BIOENGINEERING, (20 NOV 1997) Vol. 56, No. 4, pp. 441-448.
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- AU Munzert E; Heidemann R; Buntemeyer H; Lehmann J; Muthing J (Reprint)
- AN 97:789543 SCISEARCH
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- TI Prospective randomized study comparing the efficacy of bioequivalent doses of glycosylated and nonglycosylated rG-CSF for mobilizing peripheral blood progenitor cells
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- AU De Arriba, F.; Lozano, M. L.; Ortuno, F.; Heras, I.; Moraleda, J. M.; Vicente, V.
- AN 1997:177333 HCAPLUS
- DN 126:220508
- L25 ANSWER 7 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 3
- TI Site- and branch-specific sialylation of recombinant human interferon-gamma in Chinese hamster ovary cell culture
- SO BIOTECHNOLOGY AND BIOENGINEERING, (20 JUL 1997) Vol. 55, No. 2, pp. 390-398.
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- AU Gu X J; Harmon B J; Wang D I C (Reprint)
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- TI Three types of recombinant human granulocyte colony-stimulating factor have equivalent biological activities in monkeys
- SO Cytokine (1997), 9(5), 360-369 CODEN: CYTIE9; ISSN: 1043-4666
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- AN 1997:400352 HCAPLUS
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- L25 ANSWER 9 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 4
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- human interferon-gamma produced by Chinese hamster ovary cell culture using serum-free media
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- L25 ANSWER 10 OF 156 HCAPLUS COPYRIGHT 2004 ACS on STN
- TI Control of interferon-gamma glycosylation by the addition of defined lipid supplements to batch cultures of recombinant Chinese hamster ovary cells
- SO Animal Cell Technology: Basic & Applied Aspects, Proceedings of the Annual Meeting of the Japanese Association for Animal Cell Technology, 8th, Fukuoka, November 6-10, 1995 (1997), Meeting Date 1995, 339-345. Editor(s): Funatsu, Kazumori; Shirai, Yoshihito; Matsushita, Taku. Publisher: Kluwer, Dordrecht, Neth. CODEN: 64WUA2
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- L25 ANSWER 12 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 5
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- L25 ANSWER 13 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
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- SO FEMS Microbiol.Lett.; (1997) 157, 2, 279-83 CODEN: FMLED7 ISSN: 0378-1097
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- AN 1998-01164 BIOTECHDS
- L25 ANSWER 14 OF 156 HCAPLUS COPYRIGHT 2004 ACS on STN
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- AN 1997:222311 HCAPLUS
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- L25 ANSWER 15 OF 156 HCAPLUS COPYRIGHT 2004 ACS on STN
- TI Chromatographic determination of extinction coefficients of non-glycosylated proteins using refractive index (RI) and UV absorbance (UV) detectors: applications for studying protein interactions by size exclusion chromatography with light-scattering, UV, and RI detectors
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- TI Sialylation of interferon-gamma in Chinese hamster ovary cell culture; recombinant protein preparation in CHO cell culture (conference abstract)
- SO Abstr.Pap.Am.Chem.Soc.; (1997) 213 Meet., Pt.1, BIOT106
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- AU Gu X; Wang D I C
- AN 1997-11606 BIOTECHDS
- L25 ANSWER 20 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
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- AU Murphy C I; Young E
- AN 1996-08666 BIOTECHDS
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- TI Enzymatic galactosylation of sugars with in situ regeneration of nucleotide sugar;

oligosaccharide production with coenzyme regeneration, using sucrose-synthase, beta-1,4-galactosyltransferase and UDP-glucose-4-epimerase, with activator addition

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- AN 1997-01028 BIOTECHDS
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- SO JOURNAL OF ORGANIC CHEMISTRY, (03 MAY 1996) Vol. 61, No. 9, pp. 2938-2945. ISSN: 0022-3263.
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- AN 1996:335752 HCAPLUS
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- AN 96:689358 SCISEARCH
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  mouse hybridoma cell culture in a perfusion culture vessel (con
  - mouse hybridoma cell culture in a perfusion culture vessel (conference abstract)
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- AN 1996-03350 BIOTECHDS
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- L25 ANSWER 35 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE  $\dot{}$  17
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- SO TETRAHEDRON LETTERS, (18 SEP 1995) Vol. 36, No. 38, pp. 6839-6842. ISSN: 0040-4039.
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- SO SYNTHETIC COMMUNICATIONS, (1995) Vol. 25, No. 5, pp. 711-718. ISSN: 0039-7911.
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- L25 ANSWER 38 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 20
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- AN 95:751628 SCISEARCH
- L25 ANSWER 39 OF 156 HCAPLUS COPYRIGHT 2004 ACS on STN
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- SO Animal Cell Technology: Developments towards the 21st Century, [Proceedings of the Meeting], Veldhoven, Neth., Sept. 12-16, 1994 (1995), Meeting Date 1994, 391-396. Editor(s): Beuvery, E. Coen; Griffiths, J. Brian; Zeijlemaker, Wim P. Publisher: Kluwer, Dordrecht, Neth. CODEN: 62VAAP
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- AN 96:226343 SCISEARCH

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CODEN: 9996F

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- TI Synthesis of sialylated oligosaccharide compound;

oligosaccharide production or glycosylation using trans-sialidase

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- PI EP 577580 5 Jan 1994
- L25 ANSWER 50 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
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gene cloning, expression and protein engineering in e.g. Escherichia coli for use as an antiaggregant and in pancreatitis therapy

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- L25 ANSWER 51 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
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- => d ab 5,9,19,21,26,28,30,41-45,47,48,55,70,72,76,81,83,91,96,104,119
- L25 ANSWER 5 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 2

  AB Chinese hamster ovary (CHO) cells producing the recombinant glycoprotein human antithrombin III (rhAT III) were batch cultivated in a 20-L bioreactor for 13 days. Neuraminidase activity in cell-free supernatant was monitored during cultivation and free sialic acid was determined by HPLC. Neu5Ac alpha(2-->3)Gal-specific Maackia amurensis and Gal beta(1-->4)GlcNAc-specific Datura stramonium agglutinin were used for determination of sialylated and desialylated rhAT III,

respectively. A commercial test kit was used for evaluation of functional rhAT III activity. Supernatant neuraminidase as well as lactate dehydrogenase activity increased significantly during batch growth. The enhanced number of dead cells correlated with increased neuraminidase activity, which seemed to be principally due to cell lysis, resulting in release of cytosolic neuraminidase. Loss of terminally alpha(2-->3) linked sialic acids of the oligosaccharide portions of rhAT III, analyzed in lectin-based Western blot and lectin-adsorbent assays, correlated with a decrease of activity of rhAT III produced throughout long-term batch cultivation. Thus, structural oligosaccharide integrity as well as the functional activity of recombinant glycoprotein depend on the viability and mortality of the bioreactor culture, and batches with a high number of viable cells are required to guarantee production of glycoproteins with maximum biological activity. (C) 1997 John Wiley & Sons, Inc.

L25 ANSWER 9 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 4 Although serum-free media have been widely used in mammalian cell AΒ culture for therapeutic protein production, the effects of serum-substitutes on product quality have not been extensively examined. This study observed an adverse effect of Primatone RL, an animal tissue hydrolysate commonly used as a serum-substitute to promote cell growth, on sialylation of interferon-gamma (IFN-gamma) derived from Chinese hamster ovary (CHO) cell culture in both batch and fed-batch modes. In batch cultures, decreased sialylation was observed at each of the glycosylation sites (i.e., Asn(25) and Asn(97)) of IFN-gamma with the use of elevated concentrations of the peptone. Although poorest sialylation was obtained with the use of a growth-inhibiting concentration of Primatone RL, diminished sialylation was observed at the optimal peptone concentration for cell growth and product yield. Since incubation of the product in Primatone RL-supplemented acellular medium did not result in decreased sialylation, the negative effect of Primatone RL could not be attributed to extracellular desialylation of IFN-gamma by components of the peptone. In the fed-batch mode, a culture utilizing a serum-free feeding medium supplemented with Primatone RL demonstrated poorer sialylation than a similar culture not fed the peptone. The results of both the batch and fed-batch experiments indicate that the adverse effect of the peptone was not due solely to ammonia accumulation. (C) 1997 John Wiley & Sons, Inc.

ANSWER 19 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN L25 Sialylation of interferon-gamma (IFN-gamma) produced by CHO cell culture AB was monitored by reverse-phase HPLC separations of the site-specific pools of tryptic glycopeptides representing the product's 2 potential N-linked glycosylation sites (i.e. Asn25 and Asn97). The IFN-gamma displayed both site- and branch-specific differences in sialylation as the Asn25 site and the Man(alpha-1,3) branch of the predominant complex biantennary glycan structures at each site were preferentially sialylated. When the sialylation profile of IFN-gamma was analyzed throughout a suspension batch culture, sialylation at each site and branch was found to be incomplete but relatively constant until a steady decrease in sialylation was observed concurrent with loss of cell viability. The introduction of competitive sialidase-inhibitor into the culture supernatant prevented the loss of sialylation following but not prior to cell death, thus indicating that the sialic acid content of the final product was determined by both incomplete intracellular sialylation and extracellular desialylation. The influences of culture medium on sialylation were also studied. (0 ref)

ANSWER 21 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN A new method for enzymatic galactosylation of a monosaccharide or oligosaccharide, with in situ nucleotide sugar coenzyme regeneration, involves reaction with sucrose-synthase (SS, EC-2.4.1.13), beta-1,4-galactosyltransferase (GT) and UDP-glucose-4-epimerase (UDPGE,

EC-5.1.3.2), and a keto sugar or derivative is added as an activator of UDPGE. The activator is preferably dUDP-6-deoxy-D-xylohexulose, TDP-6-deoxy-D-xylo-hexulose, 6-deoxyglucosone, galactosone, allosone or glucosone, at 0.01-20 (preferably 0.1) mM, and the process is carried out as a repetitive batch operation in an ultrafiltration cell. The products are useful as precursors of sialylated or fucosylated sugars, e.g. for production of sialyl-Lewis-X or derivatives involved in cell-cell recognition. The activator reactivates UDPGE in situ, eliminating the need for repeated addition of this expensive enzyme and allowing repeated use without immobilization, and has no adverse effects on activity of the other enzymes. (36pp)

L25 ANSWER 26 OF 156 HCAPLUS COPYRIGHT 2004 ACS on STN

AB A review, with 10 refs., on the structure and a large-scale preparation of sialyloligosaccharides from egg yolk.

L25 ANSWER 28 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

L25 ANSWER 30 OF 156 MEDLINE on STN DUPLICATE 12 Insects respond to a bacterial challenge by rapidly synthesizing a diverse range of antibacterial and antifungal peptides. One of them, drosocin, a 19-residue proline-rich antibacterial peptide, was isolated from Drosophila. This peptide carries a disaccharide moiety attached to a threonine residue in mid-chain position. The present report describes the enlarged-scale chemical synthesis of drosocin, glycosylated with Gal (beta 1 --> 3) GalNAc(alpha 1 --> 0). We have studied the range of activity of the synthetic glycopeptide, of two truncated glycosylated isoforms, and of the unglycosylated L and D enantiomers. Both isolated and chemically synthesized drosocins carrying the disaccharide display the same antibacterial activity. Using circular dichroic spectroscopy we demonstrated that the O-linked disaccharidic motif did not affect the backbone conformation of drosocin. The antibacterial activity of the synthetic glycopeptide was directed against gram-negative strains with the exception of the gram-positive bacteria Micrococcus luteus. Deletion of the first five N-terminal residues completely abolished the activity of drosocin. As a first approach to the study of the mode of action of drosocin, we have synthesized a non-glycosylated D enantiomer and, using this molecule, we have shown that drosocin may act on the gram-negative bacteria through a stereospecific target.

L25 ANSWER 41 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE

A Chinese hamster ovary cell line expressing recombinant human AB interferon-gamma (IFN-gamma) was grown in a 15-1 stirred tank fermenter. N-linked carbohydrate populations associated with both Asn(25) and Asn(97) were isolated by reverse-phase HPLC separation of trypsin-digested IFN-gamma and their structure determined by matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MD) in combination with exoglycosidase array sequencing. The predominant oligosaccharide at both glycosylation sites throughout the culture was a complex biantennary structure, Gal(2)GlcNAc(2)Man(3)GlcNAc(2), which was fucosylated when attached to Asn(25) but not to Asn(97). A gradual decrease in this biantennary structure was observed, with a concomitant increase in the proportion of truncated and high-mannose glycans. These experiments demonstrate the relative stability of glycosylation during batch culture and the definitive site-specific glycosylation data that ran be obtained using MALDI-MS as a monitoring technique.

ANSWER 42 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

The control of N-glycosylation in a CHO-320 cell line that produces recombinant interferon-gamma (IFN-G) was used as a model system to study the effects on the glycosylation process of protein structure, host cell

type and cell culture conditions. CHO-320 cells were adapted to grow suspended in serum-free medium based on RPMI-1640 supplemented with cattle serum albumin (CSA), insulin, transferrin and trace element supplements. The glycoform proportions were held constant at steady-state using glucose-limited chemostat systems; at a constant dilution rate of 0.015/hr, cell growth and IFN-G were transiently improved by pulses of 3.8 mM or 5.0 mM glucose. The lipoprotein supplement ExCyte minimized glycosylation deterioration in batch culture, and partially substituted the CSA content of the medium with a fatty acid-free preparation had a similar effect. Recombinant IFN-G was routinely purified from cell culture supernatant using an anti-IFN-G immunoaffinity matrix, yielding more than 98% pure IFN-G. Oligosaccharide structures of CHO cell-derived IFN-G, the influence of host cell type on IFN-G glycosylation, and the consequences of drug efficacy were also discussed. (28 ref)

- L25 ANSWER 43 OF 156 MEDLINE on STN DUPLICATE 22 The culture environment exerts a major effect on the glycosylation pattern of recombinant human interferon-gamma (IFN-gamma) produced by Chinese-hamster ovary (CHO) cells. The recombinant IFN-gamma is heterogeneous and consists of a mixture of fully (2N), partially (1N) and non-glycosylated (ON) glycoforms, and throughout batch cultures there is a decline in the proportion of fully qlycosylated IFN-gamma. Glucose and glutamine, nutrients that are depleted early in such cultures, were prima facie candidates for causing such a shift in glycoform profile. Batch feeding of these nutrients did not prevent the decline in 2N glycoform, but the glycosylation pattern of IFN-gamma was affected by the initial glutamine concentration in the culture. Under different serum-free environments the extent of IFN-gamma glycosylation was affected by (1) the concentration of BSA, (2) the quality of BSA, (3) the lipid composition of the culture medium and (4) the presence of surfactants. Moreover, the inclusion of serum in cultures caused changes in the molecular masses of the major glycoforms, that was indicative of cleavage of the core polypeptide. The results reported emphasize the necessity of considering the effects of culture media on product quality as well as on product quantity during process optimization.
- ANSWER 44 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN L25 AΒ Engineering issues in applied mammalian cell culture are reviewed with respect to: (1) secreted products from mammalian cells (monoclonal antibody production as a model for secreted protein production, increasing the productivity of monoclonal antibody production, product quality issues, and cell culture vessels for the production of secreted mammalian cell products); (2) mammalian cells as products (factors controlling cell growth and differentiation, and culture vessel design for stem cell expansion). Engineering principles which have been applied to fermentor designs for microbial systems may be used to create mammalian cell culture vessels for quite dissimilar applications. For protein expression challenges remain in increasing specific productivity of cell lines, enabling protein-free culture on a large-scale and the control of quality aspects e.g. glycosylation heterogeneity and viral clearance. In stem cell culture, challenges remain in improving recovery during cell separation, media design and culture vessel design to ensure tight control of important factors (dissolved oxygen tension). (21 ref)
- L25 ANSWER 45 OF 156 HCAPLUS COPYRIGHT 2004 ACS on STN AB Gal $\beta$ 1,4GlcNAc  $\alpha$ 2,6-sialyltransferase (EC 2.4.99.1) catalyzes the incorporation of sialic acids at the terminal positions of glycoconjugates through a NeuAc  $\alpha$ 2,6-Gal linkage. The cDNA sequences for mouse, rat, human and chicken, along with the genomic DNA sequence, and tissue specific alternative splicing in rat have been reported. To gain a further insight into the structure and function

relationship, we attempted the large **scale** production of a recombinant **sialyltransferase** in Escherichia coli in an insol. form. The product was solubilized with urea, and renatured to give the active enzyme. The renatured enzyme was similar to the enzyme obtained from rat liver, except for its dependence on ionic strength.

L25 ANSWER 47 OF 156 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE

Fed-batch culture currently represents the most attractive choice for AΒ large scale production of monoclonal antibodies (MAbs), due to its operational simplicity, reliability, and flexibility for implementation in multipurpose facilities. Development of highly productive cell lines, maximization of cell culture longevity, and maintenance of high specific antibody secretion rates through genetic engineering techniques, nutrient supplementation, waste product minimization, and control Of environmental conditions are important for the design of high-yield fed-batch processes. Initially simple supplementation protocols have evolved into sophisticated serum-free multinutrient feeds that result in MAb titers on the order of 1-2 g/L. Limited research has been published to date on the effects of various culture parameters on potentially important quality issues, such as MAb glycosylation and stability. Although most fedbatch protocols to date have relied on relatively simple control schemes, increasingly sophisiticated algorithms must be applied in order to take full advantage of the potentially additive effects of manipulating nutrient and environmental parameters to maximize fed-batch process productivity.

ANSWER 48 OF 156 NTIS COPYRIGHT 2004 NTIS on STN L25 Specificity determinants of human acetylcholinesterase (HuAChE) towards AΒ ligands (substrate and some reversible and irreversible inhibitors) were identified by combination of site-directed mutagenesis, molecular modeling and kinetic studies with enzymes mutated in active center residues Trp86, Glu202, Trp286, Phe295, Phe297, Tyr337, Phe338 and Glu450. Thus, the anionic and hydrophobic subsites as well as the acyl pocket were identified. Enzymes with resistance to OP aging were engineered. The role of N-glycosylation in the function, biosynthesis and stability of HuAChE was examined by site-directed mutagenesis (Asn to GIn substitution) of the three potential N glycosylation sites, Asn265, Asn350 and Asn464. Large scale preparation of recombinant HuAChE was performed utilizing the microcarrier technology. Over 500 milligrams of enzyme was prepared for x-ray crystallography.

AΒ

L25 AB ANSWER 55 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN Recombinant protein glycosylation is reviewed with respect to: (1) reasons why glycosylation is significant (commercial importance, methods used to study effects of glycosylation, and the effects on protein solubility, protein stability, biological activity, pharmacokinetics and immunogenicity); (2) oligosaccharide structures (N-glycosylation and O-glycosylation); (3) glucan analysis (electrophoresis, chromatography, NMR and MS); and (4) influences on glycosylation (protein structure, host cell type, culture environment and method of cell culture). Improvements in analytical procedures offer detailed glycan analysis during or soon after host cell culture. Detailed knowledge of glycoprotein biosynthesis may aid control of glycan heterogeneity by using culture media formulations/supplements. Host cells may be enqineered for biased production of certain glycoforms. Advances in carbohydrate chemistry and recombinant glycosyltransferases may lead to construction of complex oligosaccharides, which may be grafted onto recombinant proteins made in prokaryotes. Until this time, human recombinant glycoproteins are best produced in animal cell culture. (165 ref)

ANSWER 70 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN Glycosylation is cell type-specific, and thus recombinant proteins

produced in heterologous systems are almost invariably glycosylated differently from the native form. Differences can include differences in both the number of attached glycan chains and precise glycan sequences at an individual glycosylation site. Glycosylation can influence activity, pharmacokinetics, and immunogenicity, and different glycosylation patterns may be associated with differences in the therapeutic profile. It is thus useful to analyze the glycosylation pattern of a recombinant protein at as early a stage as possible, and to compare to the native form, and to screen for determinants which interact with the immune system and lectins. The glycosylation pattern is very sensitive to culture method and variations in the extracellular environment, and it is thus important during scale-up to ensure that the glycosylation pattern is maintained. A production process is only valid if it reproducibly allows isolation of protein with a constant glycosylation pattern. It is useful to assess glycosylation on a batch-to-batch basis. ref)

- L25 ANSWER 72 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN Separation of a glycosylated 28-residue synthetic peptide from byproducts AB of the glycosylation reaction was performed by displacement chromatography in a reverse-phase system with benzyldimethylhexadecylammonium chloride as the displacer and a water/acetonitrile/phosphoric acid system. During method development using a column of internal diameter 0.46 cm problems attributed to either adsorption azeotropy or aggregation were overcome by optimizing acetonitrile concentration and operating at 55 deg. The method was scaled-up to 22 g per run on an axial compression column of internal diameter 15 cm. Compared with conventional elution chromatography conducted on a similar scale, the displacement process realized a nearly 8-fold increase in throughput with a significant reduction in solvent consumption. Details regarding process development and scale-up were presented. (0 ref)
- L25 ANSWER 76 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN Applications of aldolases and transferases in production of sialic acid AB derivatives were discussed. Sialyl-aldolase was used for production of 3-deoxy-D-manno-2-octulosonic acid. N-glycolylneuraminic acid (NeuGc) was produced on a large scale from N-qlycolylqlycosamine using sialy1-aldolase, and CMP-NeuGc (a tumor-associated antigen precursor) was produced using acylneuraminate-cytidylyltransferase (EC-2.7.7.43). 2 Pig liver sialyltransferase enzymes were purified by affinity chromatography, immobilized and used in sialyloligosaccharide production. Alpha-2,6-galactosyl- beta-1,4-N-acetylglucosaminesialyltransferase was used for sialylation of 3 different synthetic oligosaccharides. Alpha-2,3-galactosyl- beta-1,3-N-acetylgalactosaminesialyltransferase reacted with Gal-beta-1,3-GlcNAc, giving the 1st preparative synthesis of NeuAc-alpha-2,3-Gal-beta-1,3-GlcNAc (an epitope of the human pancreas adenocarcinoma tumor-associated antigen CA-50). Enzymatic synthesis seems to be the method of choice for modification of oligosaccharide structures on glycoproteins. (5 ref)
- ANSWER 81 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
  The recent development of enzyme-catalyzed reactions for the production of sugars, peptides and related substances was discussed. Topics considered included: the preparation of uncommon and aza sugars by aldolase-catalyzed aldol condensation followed by Pd-mediated reductive amination; methods for enzyme-catalyzed glycosylation using glycosyltransferase, glycosidase, transglycosidase and phosphorylase enzymes; large-scale production of oligosaccharides catalyzed by glycosyltransferases with in situ regeneration of sugar nucleotides; the coupling of glycosidase- and glycosyltransferase-catalyzed reactions for oligosaccharide production with minimal requirements for sugar nucleotide regeneration; cloning and expression of the catalytic domain

of glycosyltransferase for oligosaccharide production in Escherichia coli; the glycosyltransferase-catalyzed production of uncommon oligosaccharides such as sialyl Lewis x and sialyl Le(x) glycal; production of large peptides and their conjugates; the use of enzyme engineering to make enzymes more stable in dimethylformamide; and engineering subtilisin (EC-3.4.21.14) to catalyze ligation reactions. (58 ref)

- ANSWER 83 OF 156 HCAPLUS COPYRIGHT 2004 ACS on STN

  Recombinant human interleukin-5 (hIL-5) has been expressed at high levels and produced in large quantities in baculovirus infected Sf9 insect cells. The glycosylated protein was purified using immuno-affinity chromatog. and gel filtration. Purified hIL-5 has been crystallized using standard vapor diffusion techniques with PEG as a co-precipitant. The crystals belong to the C2 space group and diffract to 2 Å.
- ANSWER 91 OF 156 MEDLINE on STN DUPLICATE 42

  AB alpha-Neup5Ac-(2---3)-beta-D-Galp-(1---3)-D-GlcpNAc (2) and,
  alpha-Neup5Ac-(2---3)-beta-D-Galp-(1---3)-beta-D-GlcpNAcOMBn+ ++ were
  prepared on a large scale by the action of beta-D-Galp-(1---3)-D-GalpNAc
  (2---3)-alpha-sialyltransferase (partially purified from porcine liver)
  on beta-D-Galp-(1---3)-D-GlcpNAc and beta-D-Galp-(1---3)-beta-DGlcpNAcOMBn, respectively. The trisaccharide 2 is the epitope of the
  tumor-associated carbohydrate antigen CA 50, highly expressed in human
  pancreatic adenocarcinoma.
- L25 ANSWER 96 OF 156 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
- ANSWER 104 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN L25 Human recombinant interleukin-3 (IL-3) was produced by gene cloning and AB expression in bacteria (Escherichia coli HB101, DH1, MC1061, Bacillus subtilis 1A40 and Bacillus licheniformis T9), yeast (Saccharomyces cerevisiae D273-10B and Kluyveromyces lactis CBS 2360) and mammalian cells (COS, C127 (ATCC CRL 1616) and CHO-12). A low-cost production and purification scheme was designed using B. licheniformis because the protein secreted by B. licheniformis was not glycosylated and had a mol.weight of about 15,000. Vector plasmid pGB/IL-322 and plasmid pGB/IL-326 were constructed containing the alpha-amylase (EC-3.2.1.1) signal peptide fused to the sequences encoding mature IL-3 and placed downstream of a strong alpha-amylase and HpaII promoter, respectively. IL-3 (3 g) was purified from cell-free filtrate (48 l) by hydrophobic interaction chromatography on Fractogel TSK butyl 650C, 60% (NH4)2SO4 precipitation, anion-exchange chromatography on Q-Sepharose Fast Flow and concentration by ultrafiltration (twice), gel filtration on Sephacryl S100HR and ultrafiltration. The purified and formulated product entered clinical trials in November, 1989. (28 ref)
- ANSWER 119 OF 156 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN L25 AΒ A human cDNA containing the complete coding region for the lysosomal glycoprotein glucocerebrosidase (EC-3.2.1.45) was introduced into the genome of Autographa californica nuclear-polyhedrosis virus (AcNPV) downstream from the polyhedrin promoter. The recombinant virus (pAc373/GC) was cotransfected with wild-type AcNPV DNA into Spodoptera frugiperda SF9 cells using a modified calcium phosphate precipitation technique. Recombinant baculo virus containing human glucocerebrosidase cDNA was obtained, and this was plaque-purified and used to infect SF9 cells. The recombinant enzyme was characterized and found to be active in SF9 cells. High levels of glucocerebrosidase were produced; 40% of which was in the culture medium. The N-terminal amino acid sequence of the recombinant product was identical to that of mature, human placental qlucocerebrosidase. The enzyme in the culture supernatant and in the SF9 cells was glycosylated. The insect cell culture system could be used for large-scale recombinant glucocerebrosidase production, which is of clinical interest. (48 ref)

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